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Brief report
Standardization of MspI and HaeIII restriction karyotypes in cattle

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Cattle chromosomes were digested with two restriction endonucleases, Msp I and Hae III. The banding pattern induced by each enzyme allowed the identification and paring of all individual chromosomes and consequently the elaboration of the karyotype. This method is rapid and technically easy, and proved to be of great utility in cattle cytogenetic studies.

The first standard G-banded karyotype in domestic cattle (Bos taurus L., 2n 60) was established during the Reading Conference in 1976 (FORD et al. 1980). Since then cattle chromosomes have been extensively studied using G-, Q- and R- banding. Nevertheless, the elaboration of a karyotype in cattle is still a difficult task, because of the high chromosome number, the acrocentric structure of all autosomes and specially the similar banding pattern present in many chromosomes which make them difficult to identify (DI BERARDINO et al. 1990). Chromosome digestion with restriction endonucleases (RE) has been used to induce banding in many different animal species. In cattle, restriction enzymes were used mainly to characterise the structure and variability of centromeric heterochromatin (HIDAS 1995; CHAVES et al. 2000). Here, we report the results of the digestion of cattle chromosomes with two endonucleases: Msp I and Hae III. The bands induced by these enzymes allow the identification of all chromosomes, and consequently, the elaboration of the karyotype. The interest of this technique for cattle cytogeneticists is discussed.

MATERIAL AND METHODS

Twenty-one blood samples were collected from three characteristic Portuguese cattle breeds (five samples from the breed Arouquesa, nine from Alentejana and seven from Mertolenga). Peripheral blood lymphocytes were cultured according to the procedure de- scribed in CHAVES et al. (2002). GTG-bands were performed as in IANNUZZI et al. (1995). To induce RE- banding, slides were aged at 65°C for a 5 h period and then submitted to overnight restriction digestion with the endonucleases Hae III or Msp I (Gibco). A 40 ml solution made of 30U of enzyme in the buffer specified by the supplier was applied to each slide, before incubation in a moist chamber at 37°C. Control slides were incubated without enzymes. After overnight incubation the slides were washed three times with water at room temperature and air-dried. To verify the digestion effects on the chromosomes some preparations were stained with 5% Giemsa (Karyomax Giemsa, Gibco) in Sörensen's buffer for 10 min. For fixation, dry slides were placed twice in a 1 PBS solution for 5 min each before fixed in freshly- prepared 4% paraformaldehyde in 1 PBS during 10 min at room temperature. Preparations were de- hydrated for 2 min each in 70%, 90% and 100% chilled ethanol and air-dried. Slides were then denatured for 30 s in 70% deionised formamide in 2 SSC at 65°C, and dehydrated for 2 min each in 70%, 90% and 100% chilled ethanol and air-dried. Chromosomes were counterstained with DAPI and mounted in Vectorshield (Vector). Metaphases were examined under epifluorescence (Leica Dialux photomicroscope with 50W Hg-lamp), and photographed using a Kodak Ultra 400 ASA colour print film. A Zeiss Axioplan 2.Imaging with a 100W Hg-lamp, Axiocam digital camera and AxioVision software was also used. Digitalised photos were printed from Adobe PhotoShop using only contrast, overlay and colour optimisation functions that affected the whole image. To elaborate the karyotypes, chromosomes were classified and numbered according to the recommendations of DI BERARDINO et al. (2001).

RESULTS AND DISCUSSION

After restriction digestion with the endonuclease Msp I and counterstained with DAPI, characteristic bands were detected along all cattle chromosomes (Fig. 1a). These bands became particularly clear when the DAPI image was colour inverted and converted to black and

white (Fig. 1b). The pattern produced by the enzyme Msp I was specific for each chromosome, allowing the identification and pairing of all chromosome types, and consequentially the elaboration of the karyotype (Fig. 1c). Chromosome digestion with the endonu- cleases Hae III also induced in cattle metaphase a characteristic and reproducible restriction pattern with enough discrimination to allow the identification of all individual chromosomes (Fig. 2a). The bands were enhanced with the conversion of the DAPI image to black and white (Fig. 2b), facilitating the identification of each chromosome and the elaboration of the karyotype (Fig. 2c). Both endonucleases produced in cattle chromosomes a GTG-like banding (Fig. 3), although some variability was present in a few chromosomes. To establish a standardized restriction karyotype, chromosomes were paired to their homo- logue and compared to standard GTG bands (Di Berardino et al. 2001). Twenty distinct metaphases were analysed to confirm the banding pattern induced by each endonuclease. No differences were found in the pattern of RE-banding between the three cattle breeds here analysed.

The digestion of metaphase preparations withrestriction endonucleases reveals on cattle chromosomes a distinct and very reproducible G-like banding pattern with enough discrimination to allow identification and pairing of all individual chromosomes. The advantage of RE-bands is that, at the same level of chromosome condensation, it shows a higher level of definition compared to GTG-banding, making chromosome identification easier. This characteristic of RE-bands is especially important in cattle since, in this species, several chromosomes have very similar banding patterns and consequently are easy to confuse. This method provides an opportunity to use endonu- cleases for everyday chromosome identification in cattle cytogenetics.

Acknowledgements

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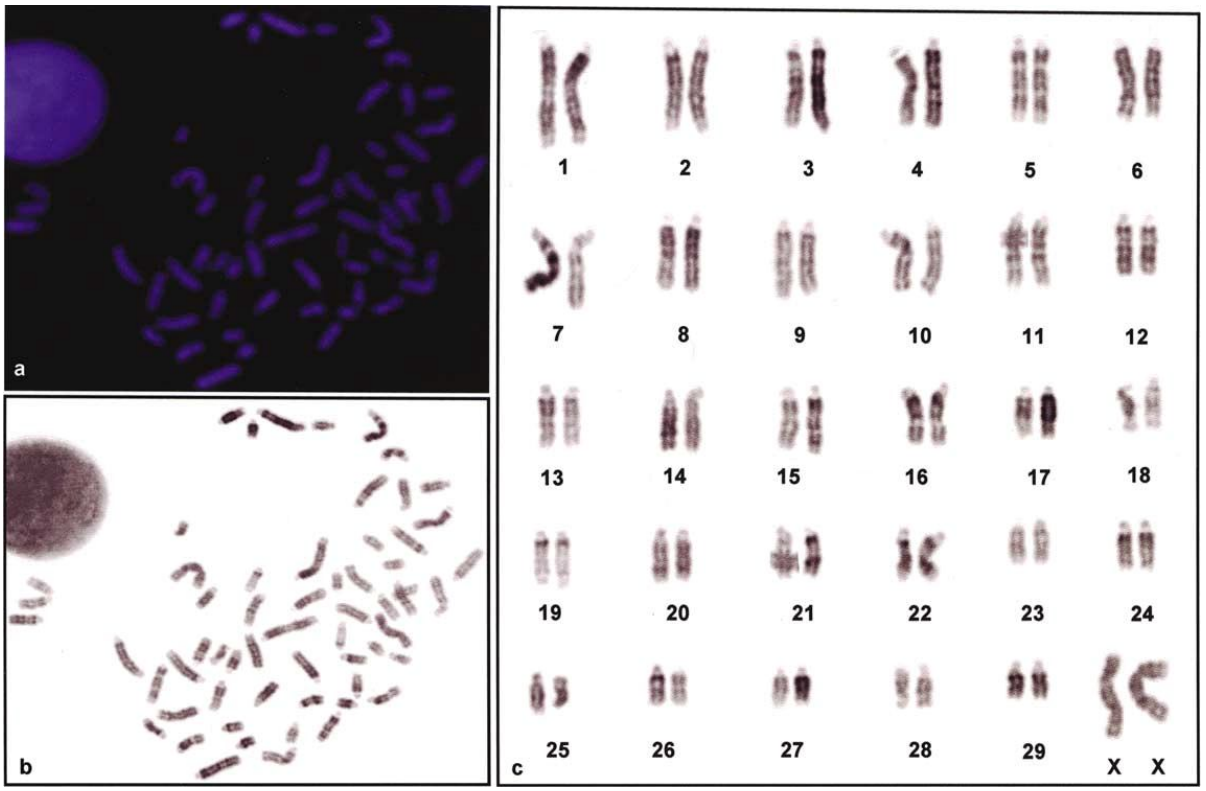

Fig. 1a c. (a) Cattle metaphase after digestion with the restriction endonuclease Msp I and counterstained with DAPI. (b) The DAPI image presented in (a) was colour inverted and converted to black and white to enhance the DAPI bands. (c) Karyotype of female cattle showing standardized banding pattern induced by the restriction enzyme Msp I.

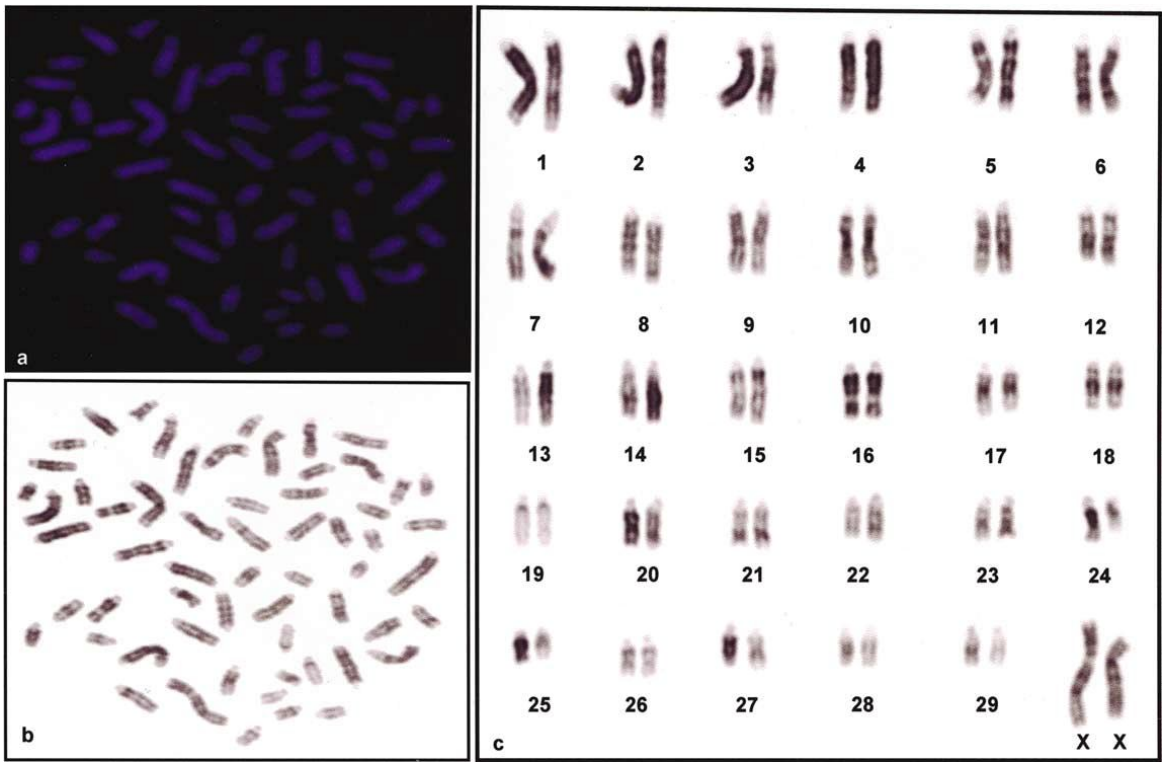

Fig. 2a c. (a) Female cattle metaphase after digestion with the restriction endonuclease Hae III and counterstained with DAPI. (b) The image presented in (a) was colour inverted and converted to black and white. (c) Karyotype showing the standardized banding pattern induced by the restriction enzyme Hae III.

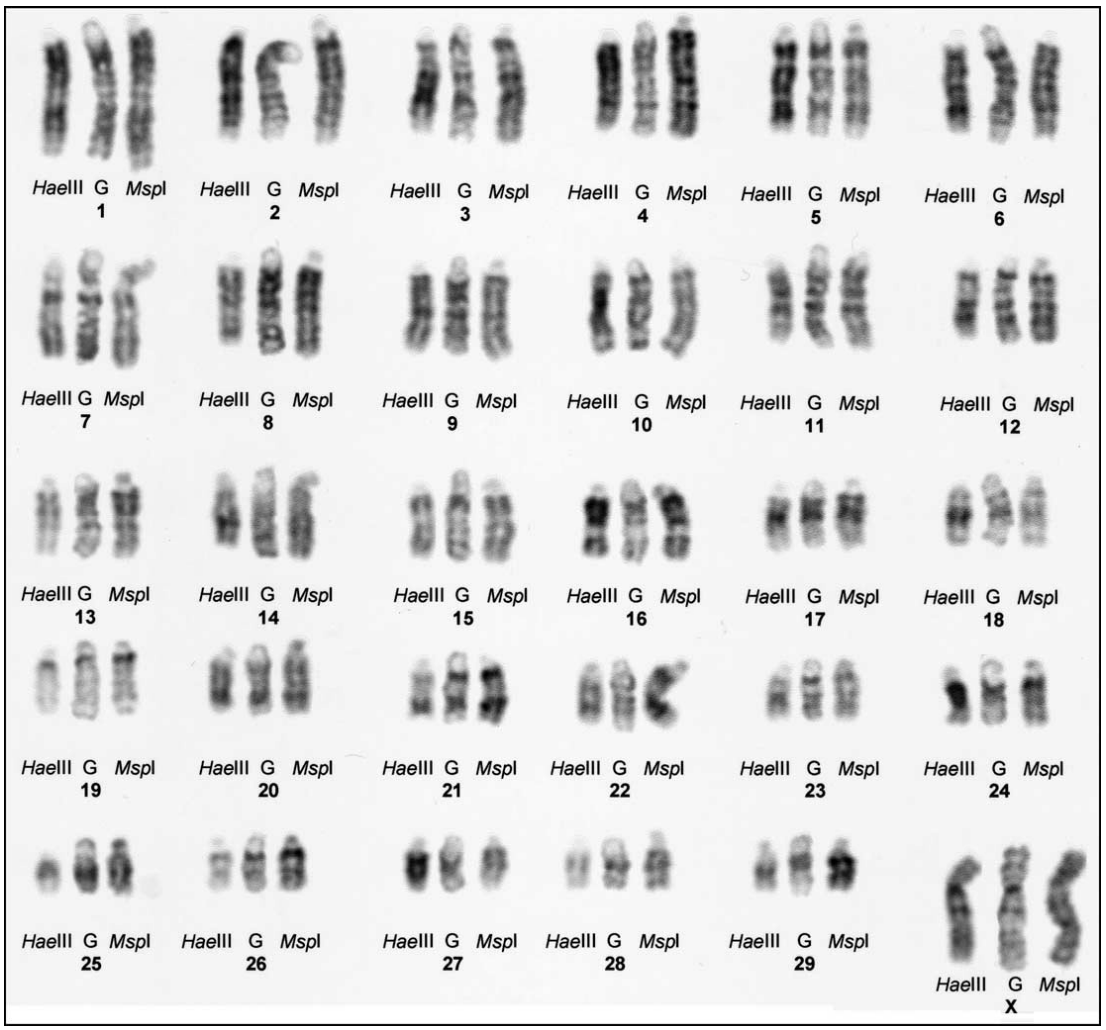

Fig. 3. Combined haploid karyotype with the banding pattern induced by the endonuclease Msp I, GTG-banded chromosomes and the bands produced by the restriction enzyme Hae III (from left to right). The letter G indicates the GTG banding pattern.